degree of hypotension and bradycardia produced in dogs, rescinnamine was twice as potent as reserpine.

The results indicate that both alkaloids are less potent than reserpine, rescinnamine or deserpidine. Shore and Brodie(10) recently found that both pseudoreserpine and raunescine reduced the serotonin content of rabbit brains but were both significantly less potent than reserpine.

Summary and conclusions. Two new alkaloids from *Rauwolfia canescens,* pseudoreserpine and raunescine have been studied for pharmacologic activity in mice and dogs. Both pseudoreserpine and raunescine have reserpine-like activity, but are less potent than reserpine. The reduction in potency has been attributed to the substitution of the methoxyl group on C-17 in ring E by a hydroxyl group.

1. Stoll, A., and Hofmann, A., *J. Am. Chem. Soc.,* 1955, v77, 820.

2. Schlittler, E., Ulshafer, P. R., Pandow, M. L., Hunt, R. M., and Dorfman, L., *Experientia,* 1955, v11, 64.

3. Klohs, M. W., Keller, F., Williams, R. E., and Kusserow, G. W., *J. Am. Chem. Soc.,* 1955, v77, 4084.

4. Hosansky, N., and Smith, E., *J. Am. Pharm. Assn.* (Sci. Ed.) 1955, v44, 639.

5. Klohs, M. W., Keller, F. E., Williams, R. E., and Kusserow, G. W., *Chemistry and Industry,* 1956, 187.

6. Toekes, I. M., *J. Pharmacol. Exp. Therap.,* 1957, v119, 354.

7. Cronheim, G., Brown, W., Cawthorne, J., Toekes, I. M., and Ungari, J., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 120.

8. Cronheim, G., and Toekes, I. M., *J. Pharmacol. Exp. Therap.,* 1955, v113, 13.

9. Gourzis, J. T., Sonnenschein, R. R., and Barden, R., *ibid.,* 1954, v85, 463.

10. Shore, P. A., and Brodie, B. B., personal communication.

Received June 7, 1957. P.S.E.B.M., 1957, v95.

Mode of Action of Aliphatic Amino Acids on Cortical Synaptic Activity. (23366)

DOMINICK P. PURPURA,* MARTIN GIRADO AND HARRY GRUNDFEST†

Departments of Neurological Surgery and Neurology, College of Physicians and Surgeons, Columbia University, N. Y. City

Electrophysiological and pharmacological evidence has disclosed that hyperpolarizing, inhibitory synapses, developing surface-positive cortical postsynaptic potentials (p.s.p.'s) occur abundantly on the superficial dendrites in the cerebral cortex, but not in the cerebellar(1,2). Recognition of this difference has facilitated analysis of the modes of action of various pharmacological agents on central synapses(1-5) that are otherwise obscured in the character of the overt response(6,7). Thus, central nervous system "stimulants" that produce convulsive activity by blockading inhibitory synapses (*e.g.* strychnine) may be distinguished(1,2) from those that activate excitatory synapses (*e.g.* Metrazol). The differential analysis is based on the absence of effect of topically applied strychnine-like agents on the cerebellar cortex, while topically applied activators of excitatory synapses produce convulsive activity in the cerebellar as well as the cerebral cortex. Differential analysis can also disclose the mode of action of "inhibitory" substances. If that overt effect results from selective blockade of excitatory synapses, topical application of the pharmacological agents to the cerebral cortex may be expected to produce inversion of the surface-negative dendritic p.s.p. to positivity as

* Scholar, Kenny Foundation. Supported in part by funds from Donner Fn. and Natl. Inst. of Neurological Diseases and Blindness.

† Supported in part by grants from Muscular Dystrophy Associations, Nat. Inst. Neurological Diseases and Blindness, Natl. Science Fn. and United Cerebral Palsy Research Fn.

the hyperpolarizing synaptic activity becomes unmasked. In the cerebellar cortex there will be only a disappearance of electrical response. This has been shown(3) to be the mode of synaptic action of topically applied γ-aminobutyric acid (GABA). The test just described does not detect directly another possible mode of inhibitory action, the selective activation of inhibitory synapses. However, this can also be analyzed by exclusion and by several indirect tests. Attention has recently been focused on the pharmacological role of GABA because this substance occurs in the brain in relatively high concentration(8,9) and because it has been identified as an active component of the "inhibitory" Factor I(10).‡ However, other ω-amino acids also exert "inhibitory" or "excitatory" effects on electrical activity of dog motor cortex. The aliphatic acids with 4 carbons or less were found to be "inhibitory" and those with 5 or more were reported to be "excitatory"(14).

Because the overt manifestations do not necessarily indicate the precise type of synaptic action, the present experiments were designed to analyze the synaptic effects of a series of ω-amino acids. A comparative study is also included of the effects of some α-amino and α,ω-diamino acids. The work included examination of their actions on evoked dendritic p.s.p.'s of cerebral and cerebellar cortex, on the simultaneously recorded electrocortical activity, and in some instances also on the direct and synaptic excitability of cortico-spinal neurons. This report will be limited to the effects produced by topical applications of the compounds. Data obtained by systemic or intraventricular injections will be described elsewhere.

Methods. The results included here are based on a series of 30 cats, all succinylcholine-paralyzed, unanesthetized preparations described earlier(1). Dendritic p.s.p.'s were evoked from the cerebral or cerebellar cortex by stimulating the cortical surface with a pair of fine (100 μ), closely spaced, teflon-insulated silver wires 0.5 to 2.0 mm distant from a stigmatic silver electrode recording from the cortex against an indifferent electrode on the frontal sinus. The responses were registered oscillographically, 2 traces of the cathode ray tube permitting simultaneous recording of independently evoked responses in different cortical regions. Bipolar or monopolar leads to a multi-channel ink writing oscillograph also recorded the "spontaneous" electrocortical activity of the same and other cortical areas. For examining the excitability of cortico-spinal neurons, one or a pair of teflon-insulated fine silver wires were inserted into the pyramidal tract to record oscillographically the descending activity resulting from stimulation of the motor cortex. In most experiments the tests were carried out in the following standardized procedure. The cortical regions under study were flushed with warm Ringer's solution and a series of 10 to 20 control responses, evoked every 2 sec. were recorded. Then 3 to 5 drops (0.1 to 0.2 ml) of the test amino acid solution (0.1 or 1.0% w/v, buffered to pH 7.4 with a synaptically inert buffer)§ were applied to the testing cerebral and cerebellar areas. Since these were generally the anterior or middle suprasylvian gyrus and the posterior surface of the vermis, only a small fraction of the applied substance remained at the tested site. An approximate index of the amount remaining was obtained by comparing the quantity of GABA applied to the cortex on filter paper with the amount applied as the fluid required to produce approximately similar effects. Thirty μg of GABA applied on a 4 mm² piece of the

‡ Several lines of evidence strongly indicate that GABA is not the only active component of Factor I. Indeed, its central nervous action probably is very restricted. GABA affects only axo-dentritic, surface synapses and primarily by topical application(3). It does not protect mice against strychnine (unpublished work in this laboratory), while certain preparations of Factor I(11), although not others(12), are powerful antagonists of strychnine. Furthermore, that portion of brain extract which does not contain free amino acids has an "inhibitory" action on crayfish stretch receptors as powerful as that of GABA (cf. 10). A peripheral synaptic action of GBA is indicated, however, by its vasomotor effects in rabbit(13).

§ Tris (hydroxy-methyl-amino-methane) does not affect electrical activity of the cortex. Other commercially available buffers, particularly those containing amino acids, were found to depress synaptic responses.

TABLE I. Cortical Synaptic Actions of Aliphatic Amino Acids.

Carbon No.	α-amino acids	ω-amino acids	α, ω-diamino acids
2	——		×
	——————Glycine——————		
3	0	—————	×
	(α-alanine)	(β-alanine)	
4	0	—————	—
	(α-aminobutyric)	(γ-aminobutyric)	(2, 4 diaminobutyric)
5	0	——	0
	(Norvaline)	(δ-amino *n*-valeric)	(Ornithine)
6	0	+ + +	+
	(Norleucine)	(ε-amino caproic)	(Lysine)
8	×	+ + + +	×
		(ω-amino caprylic)	

Symbols: — to ————— indicate increasing blockade of excitatory synapses which leads to overt "inhibitory" action. + to + + + + represent increasing blockade of inhibitory synapses leading to "excitatory" effects. 0, compound not active; ×, not available or not tried.

filter paper exerted about as much effect as 200 μg in solution dropped on the cortex. The latter mode of application was used in the testing procedure because the effects were obtained rapidly (within 30-45 sec.) and were easily reversed by flushing the cortical surface with Ringer's solution. The maximum time of action of any substance was limited to 5 min. If another agent was to be tested at the same site, the application was made only after 20 to 30 min. during which time the cortex was repeatedly flushed with Ringer's solution.

Results. Six aliphatic amino acids (C_2 to C_6 and C_8), all with the amino group on the terminal carbon (ω-compounds), were found to have synaptic action (Table I). Whereas the α-amino isomers of the C_3 to C_6 ω-compounds were inert, 2 of the 3 α,ω-diamino acids (C_4, C_6) also produced minimal synaptic effects.‖

The C_2 to C_5 ω-amino acids all had similar actions, causing a change of the evoked cerebral dendritic p.s.p.'s from surface-negativity toward surface-positivity (Fig. 1). With the standard testing procedure it was often found that the C_2 (glycine) and C_5 (δ-amino *n*-valeric acid) compounds were not able to block the depolarizing component sufficiently to unmask the hyperpolarizing, surface-positive p.s.p. This difference, however, was merely quantitative, their action in the range of concentrations used developing more slowly than did those of the C_3 and C_4 compounds. When applied in higher concentrations and for longer times, the less strongly acting C_2 and C_5 compounds reversed the surface potentials of evoked dendritic responses in the cerebral cortex. In the cerebellar cortex their action, like that of GABA(3), was the elimination of an evoked response. Although β-alanine (C_3) did not in general invert the cerebral dendritic p.s.p. as rapidly as did GABA, it nevertheless often produced a more profound surface positivity than did the C_4 substance.

The 2 longer chain ω-amino acids, C_6 (ε-amino caproic) and C_8 (ω-amino caprylic), produced entirely different effects from those of the C_2 to C_5 compounds. Both augmented the evoked dendritic p.s.p.'s of the cerebral cortex, but did not affect those of the cerebellum (Fig. 2); the differential test which indicates that the C_6 and C_8 ω-amino acids selectively block inhibitory synapses(1,2). A quantitative difference between the C_6 and C_8 compounds, as well as the qualitative difference between these 2 and the shorter chain ω-amino acids, are both strikingly shown by the relative effects on the cerebral electrocorticograms (Fig. 3), and on excitability of cortico-spinal neurons (Fig. 4).

The C_2 to C_5 compounds altered the electrocorticogram relatively little, their action—most pronounced in the case of GABA—be-

‖ Other derivatives have also been tested, and the results will be reported elsewhere.

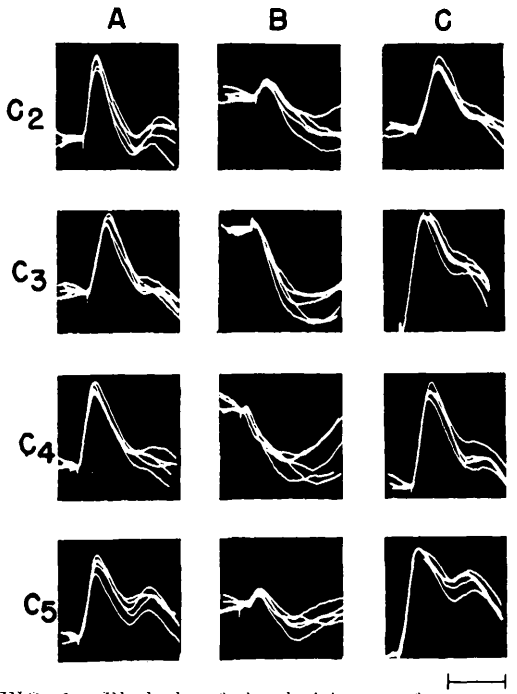

FIG. 1. Blockade of depolarizing, surface-negative dendritic p.s.p.'s in cat cerebral cortex by ω-amino acids. For illustrative purposes, all tests were performed on the same preparation. About 0.2 cc each of glycine (C_2), β-alanine (C_3), γ-aminobutyric acid (C_4) and δ-amino n-valeric acid (C_5) were applied to the cortex in 1% buffered solution at 20 to 30 min. intervals. A. Five superimposed consecutive records of the dendritic p.s.p. evoked immediately before applying the amino acid. B: 2 min. after the application. The C_2 and C_5 compounds produced less surface positivity than did the C_3 and C_4 ω-amino acids under the experimental conditions and a remnant of surface negativity is seen in both cases. C: 5 min. after flushing the cortex with warm Ringer's solution. The temporary augmentation of the dendritic surface-negativity after the C_3 to C_5 compounds was characteristic. Time, 20 msec.

ing to slow the rate and increase amplitude of the "spontaneous" activity (Fig. 3*A* to *D*). The C_6 compound, on the other hand, induced occasional paroxysmal discharges when applied for 3 to 5 min. (Fig. 3*E*). The C_8 ω-amino acid evoked violent paroxysmal discharges within 30 sec. after application of the 1% solution and this activity often progressed to sustained seizure discharges (Fig. 3*F*).

Neither the shorter chain amino acids which blockade selectively the excitatory dendritic synapses, nor the C_6 compound, which selectively blocks the inhibitory synapses, had any noticeable effect upon the excitability of cortico-spinal neurons to direct or synaptic stimulation (Fig. 4; C_4, C_6). The C_8 compound, on the other hand, progressively increased the indirect, relayed tract activity (Fig. 4; C_8), an action that is similar to the effect of strychnine, another strong inactivator of inhibitory synapses(15).

Several other results will only be mentioned briefly. Following washing out of the C_3 to C_5 ω-amino acids, there is "paradoxical" augmentation of the surface-negative dendritic p.s.p. of the cerebral cortex (Fig. 1*C*) above the control value (Fig. 1*A*). Decreased effectiveness of a given compound when it is repeatedly tested in a cycle of applications and washings is probably a tachyphylactic phenomenon. Several varieties of interaction between different compounds were also observed, but only one has been studied in detail. Although GABA itself blocks excitatory synapses its application to cortex already treated with C_6 or C_8 (Fig. 5) augments their convulsive action. This effect is increased by adding more of the latter compounds (Fig. 5*C*), but also by diluting both amino acids upon flushing the cortex with Ringer's solution (Fig. 5*F*).

Comments. The "inhibitory" and "excita-

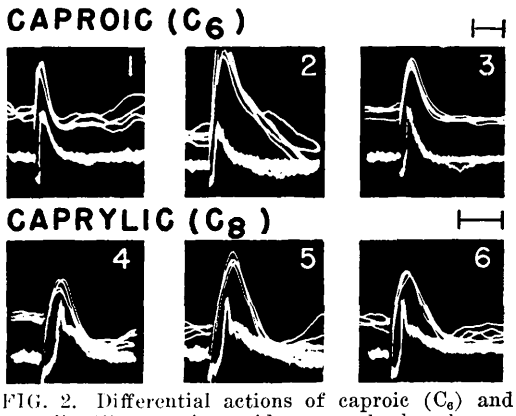

FIG. 2. Differential actions of caproic (C_6) and caprylic (C_8) ω-amino acids on cerebral and cerebellar cortex. Records for the 2 compounds taken from 2 different experiments. Direct comparison of the augmentation produced by C_6 and C_8 on the evoked p.s.p. of cerebral cortex cannot be made. 1, 4, controls; 2, 5, one min. after application of agents to cerebral (upper trace) and cerebellar cortex; 3, 6, twenty min. after frequent rinsings of the cortical sites with warm Ringer's solution. The striking augmentation of responsiveness observed in cerebral cortex (2, 5) does not occur in the cerebellar cortex. Time, 20 msec.

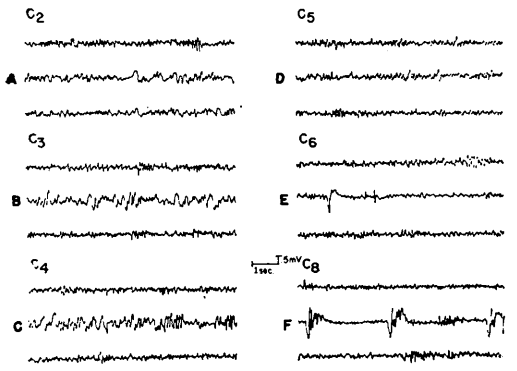

FIG. 3. Effects of topical applications of ω-amino acids on cerebral electrocorticograms. Same experiment as in Fig. 1. Bipolar leads, one on the anterior suprasylvian, the other on posterior sigmoid gyrus. In each set (A-F), a subscript (C_2 to C_8) identifies the amino acid tested. The upper record of the set was taken before applying the compound, the middle 2 min. after its application, and the lower 5 min. after flushing the cortex with warm Ringer's solution. Pronounced slowing and increased amplitude of the activity were induced maximally by the C_3 and C_4 ω-amino acids (B, C). The C_6 compound induced occasional hypersynchronous discharges (E), while the C_8 amino acid caused rhythmical high amplitude discharges and eventually, convulsive activity. In all cases the electrocortical actions were rapidly reversed.

tory" central nervous effects of the aliphatic ω-amino acids(14) were confirmed in the present work and their modes of synaptic action elucidated. Both types of effects are caused by inactivation (blockade) of synapses. The overt "inhibitory" result is caused by elimination of depolarizing, excitatory p.s.p.'s, and the compounds that produce this effect (the C_2 to C_5 substances) all act on both the cerebellar and the cerebral cortical responses, but in a different manner at the 2 structures. The "excitatory" action exerted by the C_6 and C_8 ω-amino acids is due to selective blockade of inhibitory synapses, and accordingly these compounds, like strychnine, do not produce cerebellar effects on topical application.

There is a quantitative aspect to the effects of the different ω-amino acids within each group. Among the shorter chain compounds, glycine (C2) and δ-amino *n*-valeric acid (C_5) seem to have the weakest action as blockaders of depolarizing synapses. β-Alanine (C_3) and GABA (C_4) exert almost equally a much greater action. Of the 2 longer chain ω-amino acids tested, the C_8 compound has a much more powerful selective action than does the C_6 compound, and on topical application ω-amino caprylic acid is as strongly acting as is strychnine. Like strychnine, the C_8 amino acid also affects the hyperpolarizing inhibitory synapses of the axo-somatic type. The paroxysmal action of both is associated with marked augmentation of the synaptically excited component of pyramidal tract activity after a single cortical stimulus (Fig. 4).

The various differences between the different length ω-amino acids presumably are ascribable to varieties of molecular configurations of synaptic membrane. The same factor probably also accounts for ineffectiveness of α-amino acids, and for loss of effectiveness when an α-amino group is added to an ω-amino acid (Table I). The fact that glycine, an α-compound, but with the amino group terminal, has synaptic action, like the longer

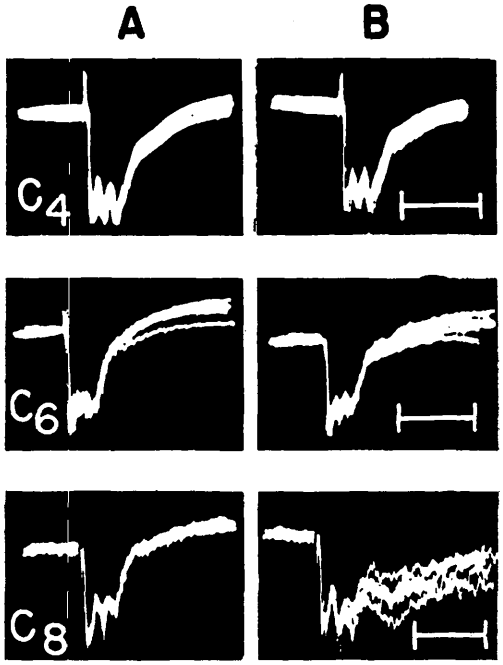

FIG. 4. Effects of C_4, C_6 and C_8 ω-amino acids on evoked activity in pyramidal tract recorded at midolivary level following motor cortex stimulation. A, control; B, 10 min. after repeated application of the solutions (0.2 cc, 10^{-2} w/v in each case) to motor cortex. Each of the agents tested in a different experiment. Ten superimposed tracings for the C_4, and 5 superimposed tracings for the C_6 and C_8 series. Significant augmentation in synaptically evoked late responses occurs only with C_8 ω-amino acid. Time, 10 msec.

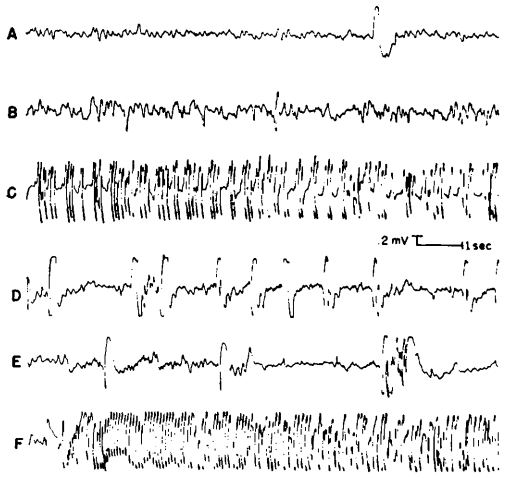

FIG. 5. Interactions of C_4-C_6 (A-C) and C_4-C_8 (D-F) ω-amino acids. A: Cerebral electrocorticogram after applying C_6; B: one min. after application of γ-aminobutyric acid; C: 30 sec. after application of additional C_6. D-F: Another experiment. D, electrocorticogram after C_8. E, one min. after γ-aminobutyric acid was added to C_8. Note some depression of convulsant action of C_8. F, 30 sec. after flushing both agents from cortex.

chain ω-amino acids, suggests that the polar separation of the carboxy and amino groups is important to the pharmacological action. Other data also show that the carboxy group is necessary in the amino acid series, but several types of more complex structures are also synaptically active, as will be reported elsewhere. Adding, as they do, a new series of compounds of analyzable synaptic action and of readily modifiable structural complexity, the data described here therefore provide new means for study of molecular organization in synaptic membrane.

Conclusion. Several types of electrophysiological tests have been applied to the analysis of the pharmacological actions of amino acids. Only the ω-amino acids have been found to have pronounced synaptic effects among the available C_2 to C_8 compounds. The α-amino acids, with the exception of glycine which has its amino group in the terminal position, are not synaptically active. Synaptic action is reduced or abolished by conversion to the α, ω-diamino compound. The ω-amino acids all act to blockade synapses, but quantitative and qualitative differences characterize the compounds of different carbon length. The C_2 to C_5 compounds block predominantly the depolarizing, excitatory synapses, while the C_6 and C_8 ω-amino acids block selectively the hyperpolarizing, inhibitory synapses. Within each group there are quantitative differences in effectiveness of selective action. The findings provide a new series of pharmacological tools for the analysis of the molecular structure and organization of central nervous synapses.

1. Purpura, D. P., and Grundfest, H., *J. Neurophysiol.*, 1956, v19, 573.

2. ———, *ibid.*, 1957, v20.

3. Purpura, D. P., Girado, M., and Grundfest, H., *Science*, 1957, v125, 1200.

4. Purpura, D. P., in *Symposium on Reticular Formation of the Brain*, in press.

5. Grundfest, H., *ibid.*, in press.

6. ———, *Ann. N. Y. Acad. Sci.*, 1957, v66, 537.

7. ———, *Physiol. Revs.*, 1957, in press.

8. Awapara, J., Landua, A. J., Fuerst, R., and Seals, B., *J. Biol. Chem.*, 1950, v187, 35.

9. Roberts, E., and Frankel, S., *ibid.*, 1950, v187, 55.

10. Bazemore, A., Elliott, K. A. C., and Florey, E., *Nature*, 1956, v178, 1052.

11. Florey, E., and McLennan, H., *J. Physiol.*, 1955, v130, 446.

12. Elliott, K. A. C., and Florey, E., *J. Neurochem.*, 1956, v1, 181.

13. Takahashi, H., Tiba, M., Iino, M., and Takayasu, T., *Jap. J. Physiol.*, 1956, v5, 334.

14. Haypashi, T., and Nagai, K., *XX Int. Physiol. Cong.*, 1956, 410.

15. Purpura, D. P., and Grundfest, H., *EEG and Clin. Neurophysiol.*, 1956, v6, 162.

Received June 13, 1957. P.S.E.B.M., 1957, v95.